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Phylogeographic Structure of the Formosan Wood Mouse, *Apodemus semotus* Thomas

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Fu-Hsiung Hsu, Fei-Jann Lin and Yao-Sung Lin (2001) Phylogeographic structure of the Formosan wood mouse, *Apodemus semotus* Thomas. *Zoological Studies* **40**(2): 91-102. Phylogeographic structure of the Formosan wood mouse, *Apodemus semotus* Thomas (Muridae), was studied by the PCR-RFLP method. In total, 271 individuals collected from 23 locations throughout its distribution range in Taiwan were examined. Eleven restriction enzymes were used to assay restriction fragment length polymorphism in a 2800-bp PCR-amplified fragment of mtDNA, representing part of the *cytochrome b* (*CYTb*) and the *control region* (*DL*). In total, forty-four mtDNA haplotypes were detected. The sequence divergence between all pairs of haplotypes ranged between 0.20% and 3.20%. Phylogenetic analysis revealed that the 4 primary mtDNA lineages have been separated by sequence divergences of 0.86% to 1.78%. The geographic structure of mtDNA diversity and the lineage distribution are complex, but each lineage reveals a limited geographic distribution from north to south in the Central Mountain Range of Taiwan. According to the geographic distribution of 7 dominant haplotypes and the locality-based UPGMA dendrogram, we resolved the northern and south-central geographical assemblages. This phylogeographic pattern of the species might have resulted from a plausible historical scenario involving isolation of its populations in several intermontane refugia, and their re-dispersion and introgression in postglacial periods during the Pleistocene.

Key words: Phylogeographic structure, mtDNA, RFLP, *Apodemus semotus*.

Macrogeographic patterns characterized by large phylogenetic separations of many species of animals are usually explained by historical events caused by glaciation in the Pleistocene (Riddle et al. 1993, Pillips 1994, Jaarola and Tegestrom 1995, Osentoski and Lamb 1995, Walker et al. 1995, Holder et al. 1999). Regional geographic localizations of closely related haplotypes are, on the other hand, usually ascribed to limited contemporary gene flow (Ball et al. 1988, Patton et al. 1994, Jaarola and Tegestrom 1996). However, it is evident that current ecology, demography, and historical patterns of vicariance and dispersal may act in concert to produce a very complex population structure (Avise 1992 1994).

Taiwan is a mountainous island with 2/3 of its area at elevations above 1000 m. The steep Central Mountain Range largely runs along the longitudinal axis of the island with the highest peak at nearly 4000 m above sea level (Chen 1993). A substantial elevational change thereby exists and creates a sharp ecological gradient. Kano (1940) studied faunistic affinity and indicated that the vertebrate fauna of Taiwan consists of 2 major elements of different geographical origins: the Oriental element confined predominantly in the lowlands and the Palaearctic element mostly in the highlands (Kuroda 1952). There are 13 species of murine and microtine rodents in Taiwan, of which 9 are mainly distributed at elevations lower than 2000 m, and the other 4 species at elevations higher than 1500 m (Lin 1982, Lin 1985, Yu 1992 1994). Therefore, the distribution of these rodents in Taiwan may be divided into 2 regions: the high-elevation region and low-elevation region. Lin (1985) proposed the elevations at 1500 to 2000 m as an interface zone between the above-

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mentioned 2 regions for mammals in Taiwan.

The high mountain range may hinder gene flow among conspecific geographical populations in the lowlands. Some phylogeographic studies have suggested that there is a possible occurrence of in situ divergence with a biogeographic event, and/or the Central Mountain Range interrupts transversally directed gene flow between eastern and western populations of lowland species of animals, enhancing their genetic differentiation (Yang et al. 1994, Chang and Liu 1997, Toda et al. 1997 1998, Yeh 1997). On the other hand, many drainages are deeply etched into the mountainous terrain, physically isolating the ridges, which may interrupt gene flow among conspecific geographical populations in the highlands. However, up to now only a few conspecific phylogeographic studies on the highland species of animals in Taiwan (Hsu et al. 2000) have been undertaken.

Hsu et al. (2000), using mtDNA markers, demonstrated the phylogeographic pattern in the endemic Formosan white-bellied rat, *Niviventer culturatus* (Thomas), which lives at elevations between 1500 and 3600 m. Unlike lowland species, *N. culturatus* shows little phylogeographic patterning among the haplotypes. It seems, counter-intuitively that the topography of the Central Mountain Range does not act as an effective barrier to prevent its gene flow. Hsu et al. (2000) considered this an indication that *N. culturatus* is a demographically "young" species with a high level of gene flow. In fact, above 1500 m, the Central Mountain Range displays contiguous suitable habitat without obvious deep river valleys dividing it. Yu (1995) also indicated that the potential isolating effect imposed by deep river valleys is minimal for *N. culturatus*. Therefore, population differentiation in highland species would be minor, except for those species with a low dispersal ability or with restricted distributions at higher elevations. However, Avise and Ball (1990) and Neigel and Avise (1993) proposed that mtDNA phylogeny resulting from historical scenarios should be favored, if (1) concordant support is obtained from other genetic traits, or (2) several unrelated taxa show congruent phylogeographic patterns.

In this study, we used a PCR-RFLP assay to examine the phylogeographic structure of mtDNA haplotypes of the Formosan wood mouse, *Apodemus semotus*. This mouse is endemic to Taiwan, and widely distributed in mountain areas at elevations of 1400-3700 m (Lin et al. 1987, Lin and Shiraishi 1992b, Yu 1992 1993 1994). It is a habitat generalist that commonly occurs in bamboo (*Yushania niitakayamensis*) grasslands, broadleaf and coniferous forests, and areas with subalpine shrubs. It has been frequently captured sympatrically with *N. culturatus* (Yu 1993). It prefers microhabitats with woody structure and heavy twig cover (Lin and Shiraishi 1992c). It is also a highly opportunistic breeder which has a potential to breed year round with a peak in both the spring and autumn (Huang 1986, Lin and Shiraishi 1992a, Yu 1993).

The objectives of this study were to determine the phylogeographical pattern of genetic variation in the Formosan wood mouse, to explain the effect of Pleistocene glaciation on its genetic structure, and to examine whether some deep drainages in the Central Mountain Range constitute geographical barriers to gene flow of this highland species.

MATERIALS AND METHODS

In total, 271 individuals of the Formosan wood mouse were collected from 23 locations in the Central Mountain Range of Taiwan at elevations above 1500 m (Fig. 1; Table 1). Small pieces of liver were

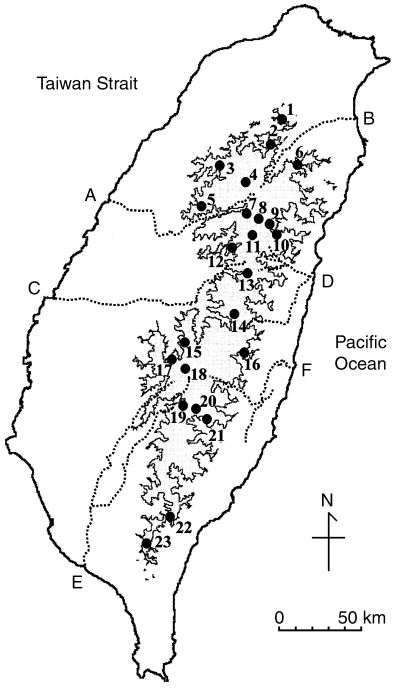

Fig. 1. Map of Taiwan showing sampling locations for the Formosan wood mouse. The shaded area indicates elevations above 1500 m; the dotted lines are some major river valleys encoded as follows: A = Tachia River, B = Lanyang River, C = Choushui River, D = Hualien River, E = Kaoping River, and F = Hsiukuluan River. The numbers 1-23 are the location codes in table 1.

dissected out as the sources of mtDNA. These tissue samples were frozen in liquid nitrogen in the field and kept in a deep freezer at –70 °C in the laboratory, or preserved in 95% ethyl alcohol and kept at ambient temperature. At the initial stage of this study, mtDNA was isolated from 0.1 to 0.5 g of liver tissue according to the protocol established by Hoelzel (1992). Later on, an adequately purified mtDNA sample was obtained using a mtDNA Extraction Kit (GeneLabs Life Science Corp., Natick, ME). The precipitated and dried mtDNA was finally resuspended in a 30-50 μl of sterile distilled water and stored at –70 °C for later use.

Symmetric amplification of the mtDNA fragment by the primers CL1 (5'-CGAAGCTTGATATGAAAA-ACCATCGTTG-3') and PB (5'-AGTGGGGTATCTA-ATCCCAG-3') was similar to that used by Hsu et al. (2000). A region of about 2800 bp of the mitochondrial genome which included the *cytochrome b* (*CYTb*) and the *control region* (*DL*) was amplified. Thermal cycling was performed with a Perkin Elmer-Cetus cycler (Foster City, CA). The sequence was amplified through 38 cycles (94 °C, 40 s; 50-55 °C, 40 s; 72 °C, 4 min) with Super *Taq* polymerase (HT Biotechnology, Cambridge, UK). All cycling began with a 'hot start' at 94 °C for 1 min, and finished with a final extension time of 10 min at 72 °C and a rapid ramp to 4 °C until the tube was removed. A 3-μl sample from each reaction was assayed by electrophoresis on a 1.2% agarose minigel with visualization under UV light after ethidium bromide staining.

In the pilot study, twenty-one restriction enzymes were tested on 44 individuals. Among them, eleven enzymes (*Aci* I, *Alu* I, *Dde* I, *Eco*R V, *Hae* III, *Hinf* I, *Hpa* II, *Mbo* I, *Rsa* I, *Sau96* I, and *Ssp* I) were identified which cut all the products at least once. Aliquots at 3-5 μl of PCR product were digested according to the manufacturer's instruction. Restriction fragments were separated on 2%-3.5% agarose gels (Nusieve agarose: agarose = 3:1) and 1 × TBE buffer at 60 V. The restriction-fragment patterns were visualized and photographed on a UV light box. Fragment size was estimated by comparison to a 100-bp DNA ladder (BRL).

Table 1. Geographic regions, sampling locations, sample sizes, and haplotype frequency of the Formosan wood mouse collected from Taiwan

Region and location code	Location (latitude; longitude)	*N*	Haplotype (*n*)
Northwestern			
1	Mt. Lala (24.42N-121.25E)	6	H08 (3), H14 (1), H24 (1), H43 (1)
2	Yuanyang Lake (24.34N-121.24E)	2	H08 (2)
3	Kuanwu (24.30N-121.06E)	20	H07 (1), H08 (12), H12 (1), H25 (3), H27 (1), H43 (2)
4	Chika (24.23N-121.16E)	3	H08 (2), H13 (1)
5	Mt. Anma (24.17N-121.01E)	21	H08 (13), H09 (1), H27 (2), H43 (5)
Northeastern			
6	Mt. Taiping (24.30N-121.31E)	6	H04 (1), H08 (4), H43 (1)
7	Mt. Fushou (24.14N-121.14E)	18	H07 (1), H08 (11), H29 (2), H32 (4)
8	Bilushi (24.13N-121.13E)	13	H04 (1), H08 (6), H25 (3), H26 (2), H29 (1)
9	Kuanyuan (24.11N-121.20E)	11	H04 (1), H07 (1), H08 (5), H29 (2), H37 (1), H41 (1)
10	Bilushenmu (24.11N-121.23E)	12	H08 (11), H44 (1)
11	Mt. Hohuan (24.08N-121.17E)	12	H07 (6), H08 (4), H11 (1), H41 (1)
12	Juiyenshi (24.07N-121.11E)	8	H07 (1), H08 (5), H10 (1), H11 (1)
Central			
13	Tunyuan (24.03N-121.12E)	6	H03 (1), H08 (1), H27 (1), H29 (1), H32 (2)
14	Haitientz (23.47N-121.10E)	3	H08 (1), H29 (1), H32 (1)
15	Chunta (23.37N-120.56E)	7	H08 (1), H15 (1), H16 (3), H19 (1), H32 (1)
16	Ruisui (23.33N-121.15E)	43	H02 (2), H04 (1), H05 (1), H06 (1), H15 (4), H16 (1), H29 (7), H30 (2), H31 (1), H32 (18), H35 (1), H39 (1), H41 (1), H44 (2)
17	Mt. Ali (23.30N-121.47E)	16	H01 (1), H05 (3), H15 (4), H16 (2), H18 (1), H32 (3), H33 (1), H40 (1)
18	Nantzushienshi (23.27N-120.54E)	15	H04 (1), H15 (5), H16 (2), H19 (1), H20 (1), H21 (1), H32 (2), H38 (1), H42 (1)
Southern			
19	Tienchih (23.16N-120.54E)	9	H15 (1), H16 (4), H28 (1), H32 (3)
20	Yakou (23.16N-120.57E)	7	H01 (1), H28 (1), H32 (2), H36 (1), H42 (2)
21	Hsiangyang (23.15N-120.59E)	14	H15 (1), H16 (2), H20 (3), H32 (6), H38 (2)
22	Hsiaokuei Lake (22.42N-120.52E)	7	H15 (3), H16 (1), H22 (1), H23 (1), H32 (1)
23	Mt. Peitawu (22.38N-120.43E)	12	H15 (3), H16 (2), H17 (2), H23 (1), H32 (1), H34 (2)

Restriction patterns produced by a given enzyme were identified with the letters A, B, C, and D in descending order according to the individual pattern's frequency. Each individual was then assigned a multi-letter code for an observed composite mtDNA haplotype based on the restriction patterns across all enzymes. Each enzyme's differences in the restriction-fragment patterns were interpreted as the presence or absence of differences in restriction sites (Dowling et al. 1990, Riddle et al. 1993, Walpole et al. 1997). Fragments of fewer than 100 bp usually were not detectable, and some hypothetical fragments were assumed in order to explain all the mutational steps (Jaarola and Tegelstrom 1996). Site data for each enzyme were summarized to construct a binary site presence-absence matrix for haplotypes and individuals, respectively. The site differences were analyzed with the computer package, REAP v4.0 (McElroy et al. 1991), to generate a sequence divergence matrix for observed haplotypes (Nei and Tajima 1981, Nei and Miller 1990) and to compute the estimates of haplotype diversity (Nei 1987), nucleotide diversity, and nucleotide divergence among locations (Nei and Tajima 1981, Nei 1987).

Phylogenetic relationships among haplotypes were inferred by either maximum parsimony or distance methods. Wagner parsimony analysis of the restriction-site data was performed using the computer program PAUP v3.1.1 (Swofford 1993) to treat a site presence or absence as a discrete binary character state. Outgroup comparison is of limited use in determining placement of the root for population-level phylogeny reconstruction (Crandall and Templeton 1993, Phillips 1994) and thus was not attempted. The analysis was conducted using the heuristic search procedure with tree bisection and reconnection (TBR) swapping, random addition, and by collapsing zero-length branches. We conducted the analysis with 100 replicates and saved all minimal trees. A single midpoint rooting consensus tree was obtained by taking the 50% majority rule consensus. The amount of phylogenetic information in the data was measured by generating 10^5 random trees with PAUP. Tables of critical g_1-values of skewness test statistics are given by Hillis and Huelsenbeck (1992). The UPGMA phenogram (Sneath and Sokal 1973) and neighbor-joining tree (Saitou and Nei 1987) were constructed among haplotypes from sequence divergence data using the MEGA computer package (Kumar et al. 1993). A dendrogram reflecting the relationship of localities was generated using the UPGMA algorithm in the computer program PHYLIP (Felsenstein 1993).

The geographic heterogeneity of haplotype frequency distributions was assessed through a randomized χ^2 analysis according to Roff and Bentzen (1989). The analysis was conducted at 2 different geographic scales; among all 23 locations, and then separately for locations representing each of 4 different regions (northwestern, northeastern, central, and southern) from north to south which are separated by the major river valleys of the Tachia, Lanyang, Choushui, Hualien, Kaoping, and Hsiukuluan Rivers (Fig. 1; Table 1). The significance level was obtained by 10^4 Monte Carlo randomizations with the computer package REAP v4.0 (McElroy et al. 1991).

RESULTS

Gel electrophoresis of the PCR products of the *CYTb-DL* mtDNA fragment revealed no size variation among individuals. The numbers of variants identified for each enzyme ranged from 3 (*Aci* I, *Eco*R V, *Hae* III, *Sau96* I) to 9 (*Mbo* I, *Rsa* I). An exception was for *Hap* II that failed to generate any cut site variants among the individuals (Table 2). The 11 restriction enzymes generated 79 inferred restriction sites, of which 41 varied among individuals, and 55 unique digestion profiles from which 44 haplotypes were resolved among the 271 completely assayed individuals (Table 2). Haplotypes differed by up to 14 restriction sites, and the pairwise sequence divergences ranged from 0.20% to 3.20% with an unweighted mean of 1.55%.

Most haplotypes were rare and highly localized. For the 44 haplotypes identified, eighteen were found in a single individual and 23 were observed at a single location (Table 2). Only 10 haplotypes were identified at more than 2 locations. Seven (H07, H08, H15, H16, H29, H32, and H43) of the 44 haplotypes represented 72.7% of all individuals examined, whereas the other 37 haplotypes were observed in less than 6 individuals. Haplotype H08 was the most common being found in 81 individuals at 15 locations. Haplotype H32 was represented by 44 individuals at 12 locations. Haplotypes H15 and H16 were represented by 22 and 17 individuals, respectively, at the same 8 locations (Tables 1, 2). According to the geographic distribution, we divided these 7 haplotypes into 2 groups, one largely confined to the northern and central regions and the other the southern and central regions. Four haplotypes (H07, H08, H29, and H43) represented by 114 individuals at 16 locations from Mt. Lala to Ruisui encompass the northern to central regions. Three haplotypes (H15, H16, and H32) were represented by 83 individuals at 12 locations from Tunyuan to Mt. Peitawu,

stretching from the central to southern regions. However, four individuals of the latter haplotype groups came from Mt. Fushou in the northern region. Geographic overlap of the 2 groups was only found in a restricted area of central Taiwan.

Parsimony analysis using PAUP generated 16 equally parsimonious trees with branch lengths of 62 steps and a consistency index of 0.487, excluding uninformative characters. A survey of the lengths of 10^5 random trees yielded a g_1-value of –0.42 ($p < 0.01$), indicating that the data set contains significant phylogenetic information (Hillis and Huelsenbeck 1992). A single 50% majority-rule consensus tree (Fig. 2) revealed 4 major genetic groups from the 44

Table 2. Forty-four mtDNA haplotypes identified from 271 individuals of the Formosan wood mouse by PCR-RFLP analysis. Letters of the haplotype description refer to digestion profiles produced by the following endonucleases (from left to right): *Aci* I, *Alu* I, *Dde* I, *Eco*R V, *Hae* III, *Hinf* I, *Hpa* II, *Mbo* I, *Rsa* I, *Sau96* I, and *Ssp* I

Haplotype code	Haplotype description											*N*	Location
H01	A	A	A	A	A	A	A	A	D	A	A	2	17, 20
H02	A	A	A	A	A	A	A	A	E	A	A	2	16
H03	A	A	A	A	A	B	A	A	C	A	A	1	13
H04	A	A	A	A	A	B	A	C	C	A	A	5	6, 8, 9, 16, 18
H05	A	A	A	A	A	C	A	A	A	A	A	4	16, 17
H06	A	A	A	A	A	D	A	A	A	A	A	1	16
H07	A	B	A	A	A	A	A	B	A	A	B	10	3, 7, 9, 11, 12
H08	A	B	A	A	B	A	A	B	A	B	B	81	1-15
H09	A	B	A	A	B	A	A	B	A	B	C	1	5
H10	A	B	A	A	C	A	A	B	A	C	B	1	12
H11	A	B	A	B	B	A	A	B	A	B	B	2	11, 12
H12	A	B	B	A	B	A	A	B	A	B	B	1	3
H13	A	B	C	A	B	A	A	B	A	B	B	1	4
H14	A	B	D	A	B	A	A	B	A	B	B	1	1
H15	A	C	A	A	A	A	A	A	B	A	A	22	15-19, 21-23
H16	A	C	A	A	A	A	A	A	B	A	B	17	15-19, 21-23
H17	A	C	A	B	A	A	A	D	B	A	A	2	23
H18	A	C	E	A	A	A	A	A	B	A	A	1	17
H19	A	D	A	A	A	A	A	A	B	A	A	2	15, 18
H20	A	D	A	A	A	B	A	C	B	A	A	4	18, 21
H21	A	D	A	A	A	B	A	C	F	A	A	1	18
H22	A	D	A	C	A	A	A	E	B	A	A	1	22
H23	A	D	A	C	A	A	A	F	B	A	A	2	22, 23
H24	A	E	A	A	B	A	A	B	A	B	B	1	1
H25	A	F	A	A	B	A	A	B	A	B	B	6	3, 8
H26	A	G	A	A	B	A	A	B	A	B	B	2	8
H27	B	A	A	A	A	A	A	A	A	A	B	4	3, 5, 13
H28	B	A	A	A	A	B	A	C	C	A	B	2	19, 20
H29	B	A	A	A	A	B	A	C	C	A	A	14	7-9, 13, 14, 16
H30	B	A	A	A	A	B	A	C	G	A	B	2	16
H31	B	A	A	A	A	B	A	C	H	A	A	1	16
H32	B	A	A	A	A	C	A	A	A	A	A	44	7, 13-23
H33	B	A	A	A	A	C	A	A	I	A	A	1	17
H34	B	A	A	A	A	C	A	G	A	A	A	3	23
H35	B	A	A	A	A	E	A	A	A	A	D	1	16
H36	B	A	F	A	A	C	A	A	A	A	A	1	20
H37	B	B	A	A	B	A	A	B	A	B	B	1	9
H38	B	C	A	A	A	A	A	A	B	A	B	3	18, 21
H39	B	H	A	A	A	C	A	H	A	A	A	1	16
H40	C	A	A	A	A	A	A	A	D	A	A	1	17
H41	C	A	A	A	A	B	A	C	C	A	A	3	9, 11, 16
H42	C	A	A	A	A	B	A	I	C	A	A	3	18, 20
H43	C	A	G	A	A	A	A	A	A	A	B	9	1, 3, 5, 6
H44	C	B	A	A	B	A	A	B	A	B	B	3	10, 16

A, B, C and D indicate descending order of the restriction pattern frequency; location numbers refer to code numbers in table 1.

haplotypes. The nucleotide divergence estimates between the 4 groups range from 0.86% to 1.78% (Table 3). Group I consists of 13 haplotypes with 111 individuals occurring mainly in the northwestern and northeastern regions. Group II consists of 10 haplotypes with 55 individuals occurring mainly in the central and southern regions. Group III consists of 6 haplotypes with 51 individuals occurring mainly in the central and southern regions. Group IV consists of 8 haplotypes with 31 individuals occurring mainly in the northeastern and central regions of Taiwan (Fig. 2). The neighbor-joining tree (Fig. 3) and the UPGMA tree (not shown) generated by the matrix of sequence divergence values from restriction-site data provide grouping patterns similar to that of the

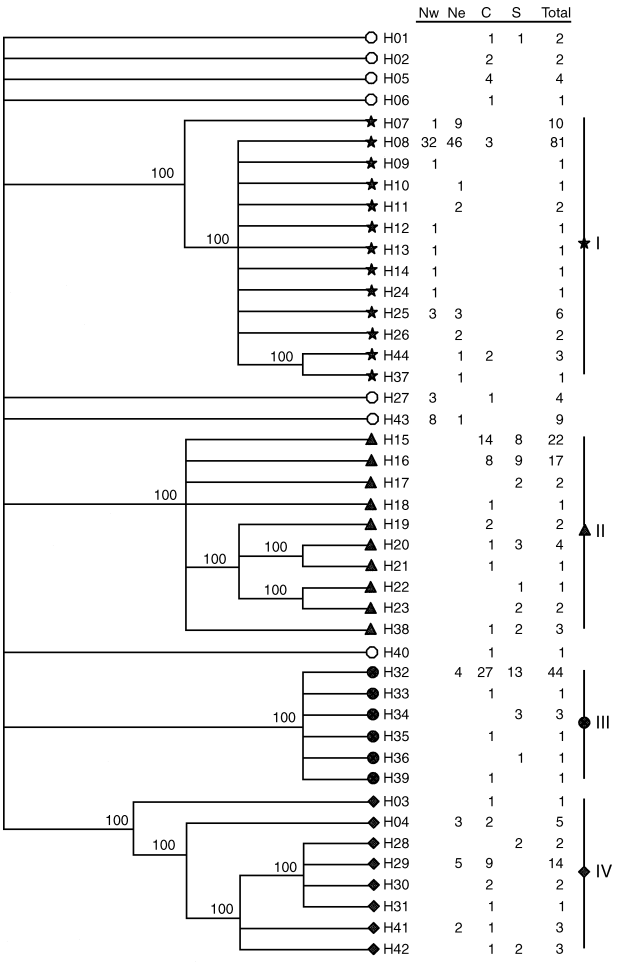

Fig. 2. Majority-rule consensus tree, representing 16 equally parsimonious trees of 62 steps, depicting the relationships among the 44 PCR-RFLP haplotypes observed in the 271 individuals of the Formosan wood mouse. The numbers on the branches indicate the value of 50% majority rule consensus in which that note was supported; H01 to H44 refer to haplotype code numbers in table 2; numbers indicate the frequency of occurrence of each haplotype in each of the 4 regions as defined in "Methods" (Nw = Northwestern, Ne = Northeastern, C = Central, and S = Southern); Roman numbers indicate different phylogenetic assemblages; haplotypes belonging to each assemblage and otherwise are indicated with different symbols.

Table 3. Estimates of mtDNA nucleotide diversity (%) (above diagonal) and divergence (%) (below diagonal) of all individuals between different mtDNA genetic groups of the Formosan wood mouse

Genetic group	I	II	III	IV
I	(0.15)	1.56	1.69	2.03
II	1.30	(0.38)	1.69	1.96
III	1.58	1.47	(0.07)	1.07
IV	1.78	1.60	0.86	(0.35)

Numbers in parentheses along the diagonal indicate the nucleotide diversity (%) within each genetic group.

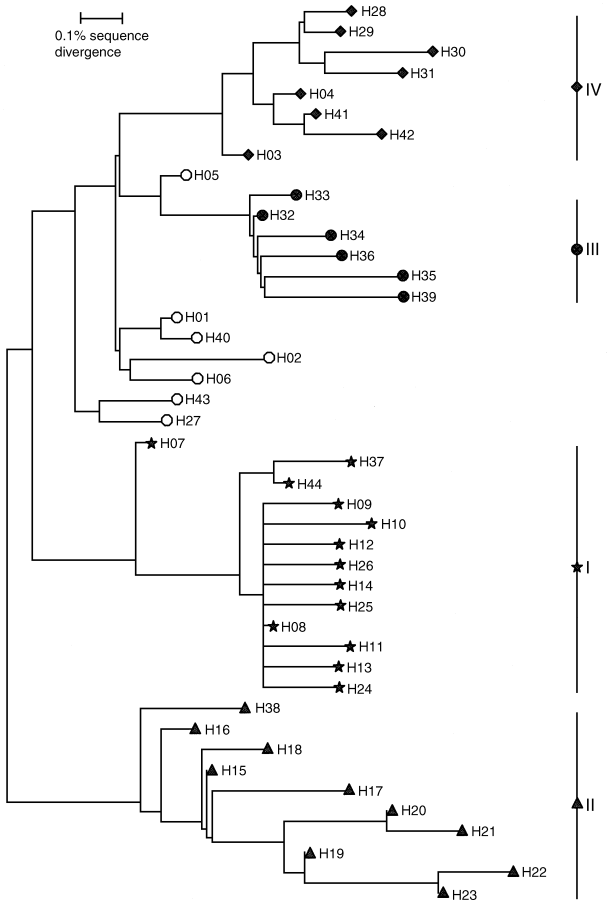

Fig. 3. Neighbor-joining tree illustrating phylogenetic relationships among 44 mtDNA haplotypes examined for the Formosan wood mouse. Haplotypes H01 to H44 refer to haplotype code numbers in table 2; Roman numbers indicate different phylogenetic assemblages inferred from the parsimony tree; haplotypes belonging to each assemblage and otherwise are indicated with different symbols.

parsimony consensus tree.

Within-location haplotype diversity ranges between 0 and 1.00 (average = 0.715) and nucleotide diversity between 0% and 1.54% (Table 4). The result of the Monte Carlo simulation suggests that a significantly heterogeneous geographic distribution of haplotypes exists across all locations (χ^2 = 1434, p < 0.0001). We also analyzed haplotype distributions separately for each of the previously identified geographic regions, nested within the sample areas. Haplotypes are distributed more homogeneously among locations within the northwestern (p = 0.2511) and central (p = 0.2047) regions. More geographic heterogeneity occurs among locations in the southern region (p = 0.0005) and a high level of heterogeneity occurs among locations in the northeastern region (p < 0.0001).

Pairwise analysis of net nucleotide divergence between locations ranges between –0.31% and 1.34%, with a mean of 0.439%. Divergence is low between the northeastern and northwestern regions, and between the central and southern regions, while it is higher between the northern and central regions, and between the northern and southern regions (Table 5). The locality-based UPGMA dendrogram (Fig. 4) also clusters locations 1-12 into the northeastern and northwestern regions, separating them from locations 13-23 into the central and southern regions. The frequency distribution of site differences between all pairs of individuals shows a bimodal distribution (Fig. 5). It is not a smooth unimodal distribution expected from a population which should have undergone a recent exponential population expansion.

DISCUSSION

The merit of animal mitochondrial DNA (mtDNA) analysis is due to its high resolution for ecological and evolutionary genetics (Avise et al. 1987, Moritz et al. 1987, Harrison 1989). It is well docu-

Table 4. Hierarchical summary of mtDNA haplotype diversity, nucleotide divergence, and distribution among locations of the Formosan wood mouse

Region and location code	*N*	Number of haplotypes	Haplotype diversity	Nucleotide divergence	χ^2 values	χ^2 distribution (10^5 reps)	*P*
Northwestern							
1	6	4	0.800	0.551			
2	2	1	0.000	0.000			
3	20	6	0.632	0.445	45.06	36.60	0.2511 ± 0.0043
4	3	2	0.667	0.135		(13.53-96.20)	
5	21	4	0.576	0.623			
Northeastern							
6	6	3	0.600	0.866			
7	18	4	0.595	0.914			
8	13	5	0.756	0.684			
9	11	6	0.800	1.079	120.11	72.90	< 0.0001
10	12	2	0.167	0.034		(46.00-117.27)	
11	12	4	0.682	0.505			
12	8	4	0.643	0.258			
Central							
13	6	5	0.933	1.019			
14	3	3	1.000	1.543			
15	7	5	0.857	0.907	144.54	126.40	0.2047 ± 0.0040
16	43	14	0.797	0.962		(67.37-215.81)	
17	16	8	0.892	0.802			
18	15	9	0.886	1.010			
Southern							
19	9	4	0.750	1.084			
20	7	5	0.905	0.947			
21	14	5	0.780	1.109	79.56	49.11	0.0005 ± 0.0002
22	7	5	0.857	0.851		(29.64-86.95)	
23	12	5	0.879	1.104			
Total			0.715	0.758	1433.62	949.49	< 0.0001
						(692.83-1312.75)	

The location codes are designated numerically as in table 1.

mented that assessing variations in mtDNA provides an effective means to uncover patterns of biogeographic discontinuity which are not clear by allozyme or morphological markers (Avise et al. 1979 1987, Avise 1989, Lamb et al. 1989). In mammals, sequence differences appear to accumulate faster in mtDNA than in many single-copy nuclear genes, allowing the resolution of lineages that have diverged from one another relatively recently (Wilson et al. 1985, Moritz et al. 1987, Hayes and Harrison 1992).

In this study, the mtDNA data obtained by the PCR-RFLP assay demonstrated a complex but structural geographic pattern in the Formosa wood mouse. The estimated genetic distances in terms of base substitutions per nucleotide site between mtDNA haplotypes are 1.55% (unweighted). The maximum-parsimony analysis reveals that there are 4 major genetic groups of the Formosan wood mouse in the Central Mountain Range of Taiwan. The neighbor-joining and UPGMA trees based on genetic distance data closely match the topology of the maximum-parsimony tree. The 4 genetic groups reveal limited geographic distributions from north to south in the Central Mountain Range of Taiwan. The geographic distribution of genetic group I shows spatial separation from genetic groups II and III. But genetic groups II and III have no associated geographic structure. Therefore, the species represents an example of the phylogeographic categories I and II as defined by Avise et al. (1987). The most likely explanation for phylogeographic category I that displays a geographic orientation involves a long-term, extrinsic barrier to gene flow. An alternative possibility is extinction of intermediate genotypes in widely distributed species with limited capabilities of dispersal and low gene flow. The plausible explanation for phylogeographic category II in which the divergent haplotype lineages occurred is a secondary admixture following the disintegration of a barrier to dispersal among geographic areas. Based on the spatial distribution of the 7 dominant haplotypes representing 72.7% of all individuals examined, we separated them into 2 groups with a partial geographic overlap in the central region of the Central Mountain Range of Taiwan. Moreover, the locality-based UPGMA dendrogram (Fig. 4) also supports the treatment of 2 sets of locality samples. The nucleotide divergence is low between the northeastern and northwestern regions, and between the central and southern regions. Within a phylogeographically structured species, a bimodal distribution usually reflects within- versus between-lineage (or region) comparisons (Avise et al. 1992, Harpending et al. 1993). Therefore, a major geographic partitioning can be depicted, i.e., the northern region and the south-central region, of phylogeography in the Formosan wood mouse. Similar phylogeographic patterns with genetic divergence were observed for the Taipei treefrog, *Rhacophorus taipeianus*, and an endemic minnow, *Zacco pachycephalus* in Taiwan (Yang et al. 1994, Wang et al. 1999). However, these 2 lowland species are restricted to western Taiwan. The northern/south-central phylogeographic separation in the Formosan wood mouse differs from the eastern/western partitioning of other lowland species, such as Swinhoe's tree lizard,

Table 5. Estimates of mtDNA nucleotide diversity (%) (above diagonal) and divergence (%) (below diagonal) of all haplotypes between different geographic regions for the Formosan wood mouse

Geographic region	Nw	Ne	C	S
Northwestern	–	0.614	1.502	1.610
Northeastern	0.016	–	1.475	1.597
Central	0.689	0.569	–	1.178
Southern	0.778	0.672	0.037	–

Key: Nw = Northwestern; Ne = Northeastern; C = Central; S = Southern.

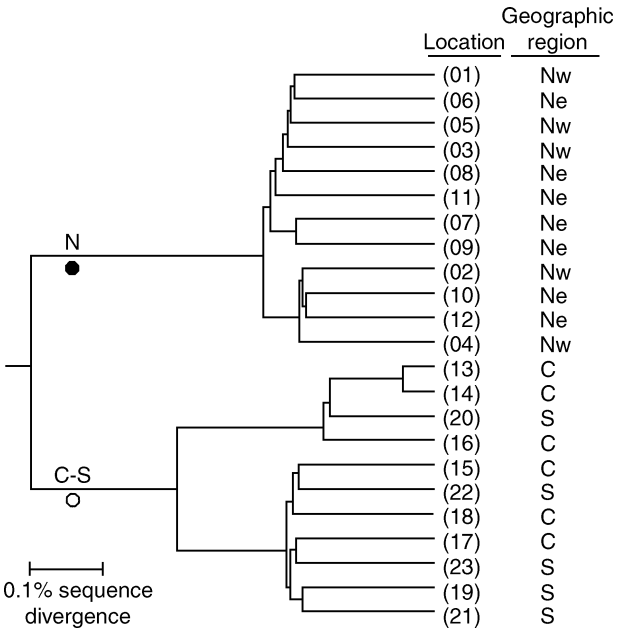

Fig. 4. UPGMA tree representing the relationships of the mtDNA nucleotide divergences among the locations sampled in this study. N and C-S on the branches refer to the 2 major lineages; numbers in parentheses denote the location code in table 1; geographic regions are denoted as follows: Nw = Northwestern, Ne = Northeastern, C = Central, and S = Southern.

Japalura swinhonis (Chang and Liu 1997), the Indian rice frog, *Rana limnocharis* (Toda et al. 1997 1998), and Moltrechti's treefrog, *Rhacophorus moltrechti* (Yeh 1997), and to limited phylogeographic variation of *N. culturatus* in the highlands (Hsu et al. 2000). However, the divergence with geographic distribution and genetic diversification of contemporary populations have probably resulted from heterogeneity of mtDNA evolution rates among different lineages, different historical vicariance or dispersal events, distinct current ecology, or differences in population structure prior to the vicariance and dispersal (Lamb et al. 1989, Avise 1992 1994, Hayes and Harrison 1992, Riddle et al. 1993, Jaarola and Tegelstrom 1995 1996, Osentoski and Lamb 1995, Walker et al. 1995, Williams and Benzie 1997).

There is no obvious geographical boundary between the northern and south-central regions in the Central Mountain Range of Taiwan. Yu (1995) concluded the potential isolating effect imposed by deep river valleys is minimal for the Formosan wood mouse. Our results of Monte Carlo simulation can not infer that major river valleys have prevented gene flow between populations. What caused the divergence between the northern and south-central regions? Based on geological evidence, Taiwan had been connected to and separated from the Asian continent several times in past geological times (Lin 1963 1966). Since its initial isolation in the late Pliocene, the island has been connected to and separated from the continent more than one time under the interactive effect of tectonic dynamics and sea-level changes (Lin 1963). Each occurrence of connecting and separating would have provided terrestrial organisms in eastern mainland Asian the opportunity to disperse into Taiwan through landbridges, and then subsequent divergence from their continental siblings due to isolation by the Taiwan Strait. We speculated as to whether the divergence of the Formosan wood mouse into the northern and south-central regions was attributable to temporal "waves" of migration. But it is difficult to envision why later-arriving colonists have been able to outcompete the resident populations, except perhaps that they dispersed from different directions (e.g., northern and southern dispersal routes). However, this scenario is hard to prove in explaining the phylogeographic divergence of the Formosan wood mouse endemic to Taiwan.

As there are no conspecific populations on the Asian continent, we have to depict the geographic partitioning of the Formosan wood mouse in Taiwan by historical events, including glacial survival in several intermontane refugia where long-term population separations have allowed the accumulation of greater mtDNA sequence differences (Avise et al. 1984, Holder et al. 1999), and postglacial re-dispersal and introgression of the populations. It may even provide an explanation for the occurrence of some of the divergent haplotypes that do not cluster with any of the 4 genetic groups. Although the estimation of the divergent time from mtDNA data is associated with errors (Hillis and Moritz 1990, Templeton 1993), we used a moderate divergence rate of 4% per 10^6 yr to represent the upper limit of the 'conventional' mammalian mtDNA clock and a lower limit for the recent rate estimates in the genus *Mus* (She et al. 1990, Prager et al. 1993, Nachman et al. 1994). The minimal and maximal times of the separation of the 4 mtDNA groups in the Formosan wood mouse are, respectively, about 215 000 and 445 000 yr ago. The average time to common ancestry within mtDNA assemblages can be estimated by π/μ, where π is the nucleotide diversity, and μ is the divergence rate (Nei and Li 1979, Avise et al. 1988). The mtDNA haplotype ancestral to the assemblages could date back 17 500 to 95 000 yr. However, Nei (1987) depicted the presence of polymorphism in the ancestral population, and the splitting of the populations characterized by the lineages may postdate the lineage separation.

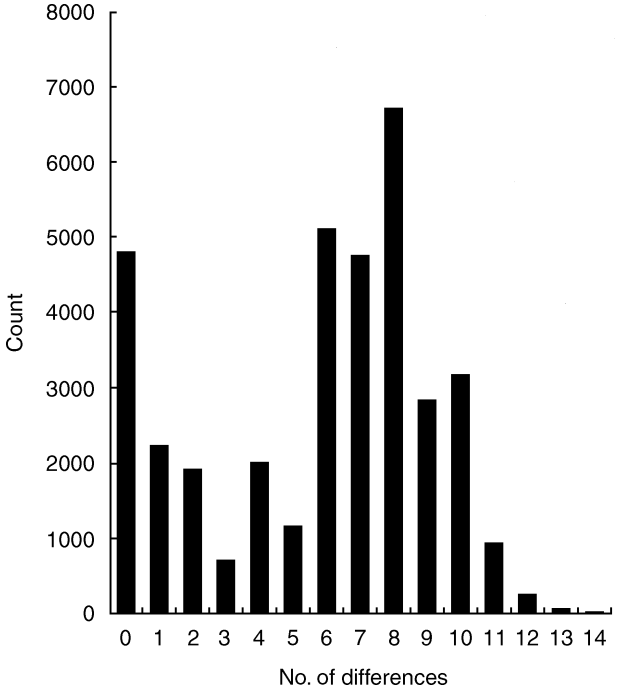

Fig. 5. Frequency distribution of pairwise site differences among individual mtDNA PCR-RFLP haplotypes of the Formosan wood mouse.

The palaeoclimatic records indicate that the dominant Pleistocene glacial-interglacial climatic cycle during the past 750 000 yr occurred at a frequency of about 10^5 yr (Hays et al. 1976). We suppose that a polymorphic and essentially panmictic population of the ancestral stock of the Formosan wood mouse invaded Taiwan before the 3rd glacial period of the Pleistocene about 240 000-150 000 yr ago (Lin 1963). The persistence of divergent lineages in intermontane refugia following the last glacial period started about 150 000 yr ago, with recent admixtures following de-glaciation about 12 500 yr ago (Lin 1963 1966). However, this is only one of the possible scenarios for the Formosan wood mouse. There is no fossil record showing when the Formosa wood mouse dispersed from mainland Asia to Taiwan. For a further understanding of the sequence divergence rate of the *Apodemus* species, it is necessary to carry out an extensive comparative survey of DNA sequence variation and genetic structures in *Apodemus* species distributed in China, Taiwan, Korea, and Japan.

The northern/south-central arrangement of genetic differentiation in the Formosan wood mouse differs from the eastern/western partition of lowland conspecific species (Chang and Liu 1997, Toda et al. 1997 1998, Yeh 1997) and the limited phylogeographic variation in *N. culturatus* of the highlands (Hsu et al. 2000). Nevertheless, the study of systematics of Taiwanese hynobiid species with limited distributions above elevation 2000 m across the Central Mountain Range shows similar geographic partitions with 5 genetic groups (Lai 1996). The highland and lowland species of Taiwan may be subjected to different geographical barriers and historical events. This study highlights the need for extensive examination of phylogeographic differentiation in other highland species of animals, therefore stimulating further discussion on comparative patterns of the current biodiversity in the highlands of Taiwan.

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REFERENCES

Avise JC. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution **43:** 1192-1208.

Avise JC. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos **63:** 62-76.

Avise JC. 1994. Molecular markers, natural history and evolution. New York: Chapman and Hall.

Avise JC, RT Alisauskas, WS Nelson, CD Ankney. 1992. Matriarchal population genetic structure in an avian species with female natal philopatry. Evolution **46:** 1084-1096.

Avise JC, J Arnold, RM Ball, E Bermingham, T Lamb, JE Neigel, CA Reeb, NC Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. **18:** 489-522.

Avise JC, RM Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surv. Evol. Biol. **7:** 45-67.

Avise JC, RM Ball, J Arnold. 1988. Current versus historical population sizes in veterbrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Mol. Biol. Evol. **5:** 331-344.

Avise JC, RA Lansman, RO Shade. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. Genetics **92:** 279-295.

Avise JC, JE Neigel, J Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. **20:** 99-105.

Ball RM Jr, S Freeman, FC James, E Bermingham. 1988. Phylogeographic population structure of red-wing blackbirds assessed by mitochondrial DNA. Evolution **85:** 1558-1562.

Chang HW, KC Liu. 1997. Biogeography and molecular phylogeny of the Swinhoe's tree lizard. *In* YS Lin, ed. Proceedings of the symposium on the conservation and management of wildlife. Taipei: Council of Agriculture, pp. 75-94. (in Chinese)

Chen CS. 1993. A geography of Taiwan. Vol. I, 2nd ed. Taipei: Nantien Books Co. 368 pp. (in Chinese)

Crandall KA, AR Templeton. 1993. Empirical tests of some predictions from coalescent theory with applications in intraspecific phylogeny reconstruction. Genetics **134:** 959-969.

Dowling TE, C Moritz, JD Palmer. 1990. Nucleic acids II: Restriction site analysis. *In* DM Hillis, C Moritz, eds. Molecular systematics. Sunderland, MA: Sinauer, pp. 250-317.

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package). Seattle: Dept. of Genetics, Univ. of Washington.

Harpending HC, ST Sherry, AR Rogers, M Stoneking. 1993. The genetic structure of ancient human populations. Curr. Anthropol. **34:** 483-496.

Harrison RG. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. TREE **4:** 6-11.

Hayes JP, RG Harrison. 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. Syst. Biol. **41:** 331-344.

Hays JD, J Imbrie, NJ Shackleton. 1976. Variations in the earth's orbit: pacemaker of the ice ages. Science **194:** 1121-1132.

Hillis DM, JP Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analysis. J. Hered. **83:** 189-195.

Hillis DM, C Moritz. 1990. An overview of applications of molecu-

lar systematics. *In* DM Hillis, C Moritz, eds. Molecular systematics. Sunderland, MA: Sinauer, pp. 502-515.

Hoelzel AR. 1992. Molecular genetic analysis of populations. New York: Oxford Univ. Press.

Holder K, R Montgomerie, VL Friesen. 1999. A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution **53:** 1936-1950.

Hsu FH, FJ Lin, YS Lin. 2000. Phylogeographic variation in mitochondrial DNA of Formosan white-bellied rat *Niviventer culturatus*. Zool. Stud. **39:** 38-46.

Huang BM. 1986. Annual reproductive cycle of the Formosan wood mouse, *Apodemus semotus*. Master's thesis, Tunghai Univ., Taiwan. (in Chinese)

Jaarola M, H Tegelstrom. 1995. Colonization history of the north European field voles (*Microtus agrestis*) revealed by mitochondrial DNA. Mol. Ecol. **4:** 299-310.

Jaarola M, H Tegelstrom. 1996. Mitochondrial DNA variation in the field vole (*Microtus agrestis*): regional population structure and colonization history. Evolution **50:** 2073-2085.

Kano T. 1940. Zoogeographic studies of the Tsugitaka mountains of Formosa. Tokyo: Institute for Ethnogeographical Researches, pp. 1-145.

Kumar S, K Tamura, M Nei. 1993. MEGA: molecular evolution analysis, version 1.02. Pennsylvania: Institute of Molecular Evolutionary Genetics, Pennsylvania State Univ.

Kuroda N. 1952. Mammalogical history of Formosa with zoogeography and bioliography. Quart. J. Taiwan Mus. **4:** 262-304.

Lai JS. 1996. Systematics of Taiwanese *Hynobius* species. Master's thesis, National Taiwan Normal Univ., Taiwan. (in Chinese)

Lamb T, JC Avise, JW Gibbons. 1989. Phylogeographic patterns in mitochondrial DNA of the desert tortoise (*Xerobates agassizi*), and evolutionary relationships among the North American tortoises. Evolution **43:** 76-87.

Lin CC. 1963. Quarternary in Taiwan: reports on historiographical studies of Taiwan. Taiwan Wen Hsien **14:** 1-92. (in Chinese)

Lin CC. 1966. An outline of Taiwan's Quaternary geohistory with a special distribution of the relation between natural history and cultural history in Taiwan. Bull. Dep. Archaeol. Anthropol. **28:** 7-44. (in Chinese)

Lin IJ. 1985. Zoogeographical studies of mammals in Taiwan. *In* YS Lin, ed. Thesis of wildlife conservation. Taipei: Dept. of Zoology, National Taiwan Univ., pp. 1-9. (in Chinese)

Lin LK. 1982. Mammals of Taiwan. Master's thesis, Tunghai Univ., Taiwan. (in Chinese)

Lin LK, PS Alexander, MJ Yu. 1987. A survey of the small mammals of Mt. Ali recreation area. Tunghai J. **28:** 669-682. (in Chinese with English abstract)

Lin LK, S Shiraishi. 1992a. Reproductive biology of the Formosan wood mouse, *Apodemus semotus*. J. Fac. Agr. Kyushu U. **36:** 183-200.

Lin LK, S Shiraishi. 1992b. Demography of the Formosan wood mouse, *Apodemus semotus*. J. Fac. Agr. Kyushu U. **36:** 245-266.

Lin LK, S Shiraishi. 1992c. Home range and microhabitat utilization in the Formosan wood mouse, *Apodemus semotus*. J. Fac. Agr. Kyushu U. **37:** 13-27.

McElroy D, P Moran, E Bermingham, I Kornfield. 1991. The restriction enzyme analysis package, version 4.0. Orono, ME: Dept. of Zoology, Univ. of Maine. 41 pp.

Moritz C, TE Dowling, WM Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. **18:** 269-292.

Nachman MW, SN Boyer, JB Searle, CF Aquadro. 1994. Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. Genetics **136:** 1105-1120.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia Univ. Press.

Nei M, WH Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Acad. Sci. USA **76:** 5269-5273.

Nei M, JC Miller. 1990. A simple method for estimating the average number of substitutions within and between populations from restriction data. Genetics **125:** 873-879.

Nei M, F Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics **97:** 145-163.

Neigel JE, JC Avise. 1993. Application of a random walk model to geographic distributions of animal mitochondrial DNA variation. Genetics **135:** 1209-1220.

Osentoski MF, T Lamb. 1995. Intraspecific phylogeography of the gopher tortoise, *Gopherus polyphemus*: RFLP analysis of amplified mtDNA segments. Mol. Ecol. **4:** 709-718.

Patton JL, MNF Da Silva, JR Malcolm. 1994. Gene genealogy and differentiation among arboreal spiny rats (Rodentia: Echimyidae) of the Amazon basin: a test of the riverine barrier hypothesis. Evolution **48:** 1314-1323.

Phillips CA. 1994. Geographic distribution of mitochondrial DNA variants and the historical biogeography of the spotted salamander, *Ambystoma maculatum*. Evolution **48:** 597-607.

Prager EM, RD Sage, U Gyllensten, WK Thomas, R Hubner, CS Jones, L Noble, JB Searle, AC Wilson. 1993. Mitochondrial DNA sequence diversity and the colonization of Scandinavia by house mice from East Holstein. Biol. J. Linn. Soc. **50:** 85-122.

Riddle BR, RL Honeycutt, PL Lee. 1993. Mitochondrial DNA phylogeography in northern grasshopper mice (*Onychomys leucogaster*) — the influence of Quaternary climatic oscillations on population dispersion and divergence. Mol. Ecol. **2:** 183-193.

Roff DA, P Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample sizes. Mol. Biol. Evol. **6:** 539-545.

Saitou N, M Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406-425.

She JX, F Bonhomme, P Boursot, L Thaler, F Catzeflis. 1990. Molecular phylogenies in the genus *Mus*: comparative analysis of electrophoretic, scnDNA hybridization, and mtDNA RFLP data. Biol. J. Linn. Soc. **41:** 83-103.

Sneath PHA, RR Sokal. 1973. Numerical taxonomy. San Francisco: WH Freeman.

Swofford DL. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1.1. Champaign, IL: Illinois Natural History Survey.

Templeton AR. 1993. The "Eve" hypotheses: a genetic critique and analysis. Am. Anthropol. **95:** 51-72.

Toda M, M Nishida, M Matsui, KY Lue, H Ota. 1998. Genetic variation in the Indian rice frog, *Rana limnocharis* (Amphibia: Anura) in Taiwan, as revealed by allozyme data. Herpetologica **54:** 73-82.

Toda M, M Nishida, M Matsui, GF Wu, H Ota. 1997. Allozyme variation among East Asian populations of the Indian rice frog, *Rana limnocharis* (Amphibia: Anura). Biochem. Syst.

Ecol. **25:** 143-159.
Walker D, VJ Burke, I Barak, JC Avise. 1995. A comparison of mtDNA restriction site vs. control region sequence in phylogeographic assessment of the musk turtle (*Sternotherus minor*). Mol. Ecol. **4:** 365-373.
Walpole DK, SK Davis, IF Greenbaum. 1997. Variation in mitochondrial DNA in populations of *Peromyscus eremicus* from the Chihuahuan and Sonoran deserts. J. Mammal. **78:** 397-404.
Wang HY, MP Tsai, MJ Yu, SC Lee. 1999. Influence of glaciation on divergence patterns of the endemic minnow, *Zacco pachycephalus*, in Taiwan. Mol. Ecol. **8:** 1879-1888.
Williams ST, JAH Benzie. 1997. Indo-West Pacific patterns of genetic differentiation in the high-dispersal starfish, *Linckia laevigata*. Mol. Ecol. **6:** 559-573.
Wilson AC, RL Cann, SM Carr, G Matthew, UB Gyllensten, KM Helm-Bychowski, RG Higuchi, SR Palumbi, EM Prager, RD Sage, M Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. **26:** 275-400.
Yang YJ, YS Lin, JL Wu, CF Hui. 1994. Variation in mitochondrial DNA and population structure of the Taipei treefrog *Rhacophorus taipeianus* in Taiwan. Mol. Ecol. **3:** 219-228.
Yeh WS. 1997. Phylogeographic structure of *Rhacophorus moltrechti*. Master's thesis, National Taiwan Univ., Taiwan. (in Chinese)
Yu HT. 1992. An elevational transect study of the distribution of small mammals in Taiwan. PhD dissertation, Univ. of California at Berkeley, CA.
Yu HT. 1993. Natural history of small mammals of subtropical montane areas in central Taiwan. J. Zool. Lond. **231:** 403-422.
Yu HT. 1994. Distribution and abundance of small mammals along a subtropical elevational gradient in central Taiwan. J. Zool. Lond. **234:** 577-600.
Yu HT. 1995. Patterns of diversification and genetic population structure of small mammals in Taiwan. Biol. J. Linn. Soc. **55:** 69-89.

臺灣森鼠(*Apodemus semotus* Thomas)之地理類緣關係

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本研究利用 PCR-RFLP 的方法來分析臺灣地區 23 個族群， 271 隻臺灣森鼠(*Apodemus semotus* Thomas)之粒線體 DNA 的地理類緣關係。我們利用 11 種不同的限制酶 (restriction enzyme) 來分析其粒線體 DNA 之 *CYTb-DL*(約 2800 bp)基因片段的限制酶片段多型性(RFLP, restriction fragment length polymorphism)。結果獲得 44 個粒線體 DNA 的單倍基因型 (haplotype) ，各單倍基因型的序列差異介於 0.20% 至 3.20% 之間。透過類緣關係的分析，我們可以發現有四個主要的分枝群，各分枝群的序列差異介於 0.86% 至 1.78% 之間。雖然這四個分枝群的基因歧異度和地理分布頗為複雜，但仍可發現各分枝群都侷限分布在由北至南的特定地理區域中。藉由七個主要單倍基因型的地理分布及各族群 UPGMA 的分析，我們可以將所有的臺灣森鼠族群區分成北部及中南部山區等兩個地理族群。我們推測這可能是臺灣森鼠受到更新世冰期的影響，移往山區間的避難所，而在冰期過後各族群再度擴散至各山區而相互混合的結果。

關鍵詞：地理類緣關係，粒線體 DNA ，限制酶片段多型性，臺灣森鼠。

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